## The molecular weight of $\alpha_{s1}$-casein B

Most molecular weights obtained for $\alpha_{s1}$-casein B are in the range 24000–29000 (see refs. 1–5). However, since a molecular weight of 16500 was recently obtained in glycine–sodium hydroxide buffer (pH 12.2, $I = 0.5$, 2.5°)[2], it was decided to redetermine the molecular weight of a single sample, using both glycine buffer and aqueous guanidine hydrochloride, which is widely used for molecular weight determinations, to effect dissociation. The results obtained indicate that $\alpha_{s1}$-casein is, under certain conditions, degraded in alkaline glycine buffer.

$\alpha_{s1}$-Casein B was prepared by a method described by THOMPSON *et al.*[6]. The sedimentation equilibrium experiments were performed in a Spinco Model E ultracentrifuge* equipped with interference optics, using the method of YPHANTIS[7]. Fringe displacements were plotted against the square of the radial distance, $r$. The molecular weight was obtained by use of the following relationship[7]:

$$\frac{\mathrm{d}\ln f}{\mathrm{d}(r^2)} = M_w(1-\bar{\nu}\rho)\cdot\frac{\omega^2}{2RT} \qquad (1)$$

where $f$ is the fringe displacement, $M_w$ is the weight-average molecular weight at the point $r$, $\bar{\nu}$ is the partial specific volume of the solute, $\rho$ is the solution density, $\omega$ is the angular velocity, $R$ is the gas constant in c.g.s. units and $T$ is the absolute temperature. Extrapolation of $M_w$ to the cell bottom yields the z-average molecular weight ($M_z$) of a mixture. The value of the partial specific volume of proteins in concentrated aqueous guanidine hydrochloride is somewhat uncertain at present. For several proteins the value is approx. 0.02 ml/g less than the value obtained in dilute salt solution[8–10]. However, SCHACHMAN AND EDELSTEIN[11] have presented evidence that for aldolase the value is greater, by 0.03 ml/g, in aqueous guanidine hydrochloride. Accordingly, we made estimates of the molecular weight of $\alpha_{s1}$-casein B by using the value of $\bar{\nu}_2$ of 0.728 ml/g found by MCMEEKIN, GROVES AND HIPP[12], as well as the values 0.71 and 0.76 ml/g.

* It is not implied that the U.S. Department of Agriculture recommends the above company or its products to the possible exclusion of others in the same business.

Preliminary molecular weights showed no significant change when the guanidine hydrochloride concentration was varied from 2 to 4 molar. The values obtained in 3 M guanidine hydrochloride are given in Table I.

TABLE I

MOLECULAR WEIGHT OF $\alpha_{s1}$-CASEIN B

Solvent: 3 M guanidine hydrochloride, 0.023 M sodium phosphate (pH 7.0, 25.0°); speed: 35600 rev./min.

| *Initial concentration* (*g/l*) | *Column height* (*mm*) | *Mol. wt.* ($M_z$) | | |
|---|---|---|---|---|
| | | $\bar{v}_2 = 0.71$ | $\bar{v}_2 = 0.728$ | $\bar{v}_2 = 0.76$ |
| 0.149 | 2.73 | 24 500 ± 700 | 26 700 | 31 800 |
| 0.149 | 2.96 | 24 600 ± 700 | 26 800 | 31 900 |
| 0.149 | 3.16 | 25 100 ± 700* | 27 400 | 32 600 |
| 0.149 | 3.40 | 24 300 ± 700 | 26 500 | 31 500 |
| 0.298 | 3.17 | 24 100 ± 700 | 26 300 | 31 300 |

* The solvent also contained 0.05 M 2-mercaptoethanol.

All of the plots of ln $f$ *vs.* $r^2$ were linear, suggesting homogeneity of molecular weight. That the molecular weight showed no significant change even when the column height was varied from 2.7 mm to 3.4 mm offered more evidence for purity and molecular weight homogeneity of the sample[7].

The molecular weight of our sample lies in the range 25000–31000, which is in agreement with previously determined values[1,3–5]. A more precise estimate will be made from our data after studies, now in progress, of the binding of guanidine hydrochloride to $\alpha_{s1}$-casein B, are completed.

The molecular weight found in this study is much larger than the value, 16500 ± 400 of SCHMIDT AND PAYENS[2], who used the solvent glycine–sodium hydroxide (pH 12.2, $I = 0.5$, 2.5°). When their conditions were used to make a molecular weight determination on our sample, the plot of ln $f$ *vs.* $r^2$ was curved upward near the base of the column, and the slope showed a reduction in molecular weight. $M_w$ throughout most of the cell was about 14000, a value similar to that obtained by SCHMIDT AND PAYENS[2].

A control on the above experiment was performed as follows: $\alpha_{s1}$-casein was exposed to glycine buffer at 2.5° for only 4.5 h, dialyzed *vs.* water and subsequently lyophilized. It was dissolved in 3 M guanidine hydrochloride and dialyzed against that solvent. The z-average molecular weight of this material was 24500 ($\bar{v}_2 =$ 0.71 ml/g). However, when a portion of the protein was dialyzed *vs.* glycine buffer at 5° for 7 days, the molecular weight (measured at 2.5°) after this time was 13500 ± 1400 (Table II). When this solution was then dialyzed exhaustively against the 3 M guanidine solvent, the molecular weight was 12500–16000 (Table II) in contrast to the molecular weight 25000 found, in that solvent, for protein that had not been previously exposed to high pH glycine. Thus, glycine buffer (pH 12.2) causes a time-dependent change in $\alpha_{s1}$-casein B.

Our results show that $\alpha_{s1}$-casein B is not stable in alkaline glycine buffer for a period of one week or less, but it is stable in concentrated aqueous guanidine hydro-

TABLE II

MOLECULAR WEIGHT OF $\alpha_{s1}$-CASEIN B EXPOSED TO HIGH pH

| *Initial concentration (g/l)* | *Solvent* | *Speed (rev./min)* | *Column height (mm)* | *Mol. wt. ($M_z$)* |
|---|---|---|---|---|
| 0.15 | Glycine–NaOH (pH 12.2, 2.5°, $I = 0.5$) | 35 600 | 2.5 | 13 500 ± 1400 (S.E.)* |
| 0.25 | Glycine–NaOH (pH 12.2, 2.5°, $I = 0.5$) | 29 500 | 6.6 | 14 000** approx. |
| 0.15 | 3 M Guanidine–HCl, 0.023 M sodium phosphate (pH 7.0, 25°) | 37 020 | 7.0 | 12 500–16 000*** |
| 0.15 | Sodium phosphate (pH 12.3, 25°, $I = 0.19$) | 33 450 | 2.4 | 28 000 ± 2500 (S.D.) |

* Average of three values obtained in same run using a six hole Kel-F centerpiece; $(1-\nu\rho) = 0.30$ (see ref. 2).

** Sample was in a double-sector carbon-filled Epon centerpiece; plot of $\ln f$ *vs.* $r^2$ showed upward curvature in region very close to the cell bottom, indicating the presence of higher molecular weight material.

*** After initial exposure to pH 12.2 glycine buffer at 5° for 7 days. Estimated on basis of $\bar{\nu}_2 = 0.71$–$0.76$ ml/g.

chloride. We do not know why the protein is unstable in the glycine buffer but use of that solvent is contraindicated for molecular weight determinations. The low molecular weight of 16 500 obtained by SCHMIDT AND PAYENS[2] would appear to be artifactual. At any rate more work is needed to prove the existence of a subunit of $\alpha_{s1}$-casein B.

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